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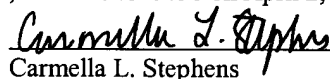
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PATENT

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ENCEPHALOPATHIES
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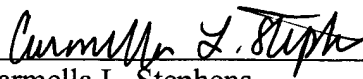
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Submitted herewith is a certified copy of PCT/EP02/10063 to which priority was
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Respectfully submitted,

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Attestation

**Les documents fixés à cette attestation
sont conformes à la version initialement
déposée de la demande de brevet inter-
national spécifiée à la page suivante.**

25 MAR 2004

Mrs. T. Bröcker-Tazelaar

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ENCEPHALOPATHIES

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V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	<p>AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT</p> <p>EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT</p> <p>EP: AT BE BG CH&LI CY CZ DE DK EE ES FI FR GB GR IE IT LU MC NL PT SE SK TR and any other State which is a Contracting State of the European Patent Convention and of the PCT</p> <p>OA: BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT</p>
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	<p>AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW</p>
V-5	Precautionary Designation Statement In addition to the designations made under Items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under Item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.	
V-6	Exclusion(s) from precautionary designations	NONE
VI-1	Priority claim of earlier national application	
VI-1-1	Filing date	05 September 2001 (05.09.2001)
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VII-1	International Searching Authority Chosen	European Patent Office (EPO) (ISA/EP)

- 1 -

DIAGNOSTIC METHOD FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

5 BACKGROUND OF THE INVENTION

Field of the invention

10 This invention relates to a diagnostic method for a transmissible spongiform encephalopathy (TSE).

Description of the related art

15 Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases of the central nervous system. They can be transmitted, inherited or occur sporadically and are observed in animals, e.g. as bovine spongiform encephalopathy (BSE) in cattle or scrapie in sheep, as well as in humans as Creutzfeldt-Jakob disease (CJD), Gerstman Sträussler Scheinker syndrome, Fatal Familial Insomnia or Kuru. They have a long incubation period, leading to ataxia, dementia, psychiatric disturbances
20 and death. Neuropathological changes include vacuolar degeneration of brain tissue, astrogliosis and amyloid plaque formation. The diseases are difficult to diagnose pre-mortem.

The cerebrospinal fluid (CSF) of CJD patients displays two additional polypeptides
25 (known as 14-3-3 polypeptides) by two-dimensional polyacrylamide gel electrophoresis [Harrington, M.G. New England Journal of Medicine 315, 279 (1986), Hsich, G., Kenney, K., Gibbs, C.J., Lee, K.H. & Harrington, M. B. New England Journal of Medicine 335, 924 (1996).] The function of these 14-3-3 polypeptides remains unclear in TSE. They can be used in a pre-mortem test for CJD
30 diagnostic evaluation, but have low specificity.

Monoclonal antibodies to the abnormal form of prion protein (which is associated with CJD) are available and can be used in an enzyme-linked immunoassay, as described in PCT Specifications WO 98/23962 and 98/32710 and Schmerr, M.J., the Beckman Coulter Pace Setter Newsletter 3(2),1-4 (June 1999), but these procedures
5 have not yet been fully developed.

PCT/EP 01/02894 relates to a diagnostic assay for TSEs in which the concentration of heart or brain fatty acid binding protein (H-FABP or B-FABP) is determined in a sample of body fluid.

10

US-A-6225047 describes the use of retentate chromatography to generate difference maps, and in particular a method of identifying analytes that are differentially present between two samples. One specific method described therein is laser desorption mass spectrometry.

15

WO 01/25791 describes a method for aiding a prostate cancer diagnosis, which comprises determining a test amount of a polypeptide marker, which is differentially present in samples of a prostate cancer patient and a subject who does not have prostate cancer. The marker may be determined using mass spectrometry, and
20 preferably laser desorption mass spectrometry.

25

Development of new non-invasive TSE markers for body fluids (in particular, CJD and BSE markers in blood) and new methods of determining the markers would help clinicians to establish early diagnosis. This problem has now been solved by the present invention.

SUMMARY OF THE INVENTION

The present invention provides a method of diagnosis of a transmissible spongiform encephalopathy (TSE) or the possibility thereof in a subject suspected of suffering
5 from the TSE, which comprises subjecting a sample of body fluid taken from the subject to mass spectrometry, thereby to determine a test amount of a polypeptide in the sample, wherein the polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects, and has a molecular weight in the range of from 3500 to 30000; and determining whether the test amount is
10 consistent with a diagnosis of TSE.

The invention also provides use of a polypeptide which is differentially contained in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular weight in the range of from 3500 to 30000 and being determinable by
15 mass spectrometry, for diagnostic, prognostic and therapeutic applications.

The invention further provides a kit for use in diagnosis of TSE, comprising a probe for receiving a sample of body fluid, and for placement in a mass spectrometer, thereby to determine a test amount of a polypeptide in the sample, wherein the
20 polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects, and has a molecular weight in the range of from 3500 to 30000.

BRIEF DESCRIPTION OF THE DRAWINGS

25 Figure 1 is a spectral view of CSF from normal and CJD-infected samples using laser desorption/ionization mass spectrometry;

Figure 2 is a corresponding view highlighting a protein peak at about 4780 Da in
30 CJD-infected CSF samples;

Figure 3 is a corresponding view highlighting protein peaks at about 6700 and 8600 Da in CJD-infected CSF samples;

- 5 Figure 4 is a corresponding view highlighting a protein peak at about 13375 Da in CJD-infected CSF samples;

Figure 5 is a spectral view of plasma from normal and BSE-infected samples using laser desorption/ionization mass spectrometry;

10

Figure 6 is a view corresponding to Figure 5 and highlighting a protein peak at about 10220 Da in BSE-infected plasma samples;

- 15 Figure 7 is a spectral view of plasma from CJD-infected patients (CJD+) and non-infected patients (CJD-) using laser desorption/ionization mass spectrometry;

Figures 8A and 8B are views corresponding to Figure 7 and highlighting polypeptide peaks that are differentially expressed in the CJD+ and CJD- plasma samples; and

- 20 Figures 9A to 9E are spectral views of plasma from CJD-infected patients (plasma CJD) and non-infected patients (plasma CTS) highlighting further polypeptide peaks that are differentially expressed in the infected and non-infected samples.

DESCRIPTION OF PREFERRED EMBODIMENTS

25

The invention provides a method of diagnosis of a transmissible spongiform encephalopathy (TSE) or the possibility thereof in a subject suspected of suffering from the TSE. A sample of body fluid taken from the subject is subjected to mass spectrometry, to determine the presence or absence in the sample of a polypeptide marker which is differentially contained in the body fluid of TSE-infected subjects and non-infected subjects. The polypeptide marker has a molecular weight in the range of from 3500 to 30000, preferably from 3900 to 18000, and the presence or absence of the marker is indicative of TSE.

30

The method is applicable to all types of TSE, and to any human or animal suffering or suspected of suffering therefrom. The method is especially applicable to the diagnosis of CJD, especially new variant CJD, in human patients, and to BSE in ruminant
5 animals such as cattle, and to BSE-like diseases in other animals, such as scrapie in sheep.

The term polypeptide includes proteins and protein fragments, as well as peptides modified by the addition of non-peptide residues, e.g. carbohydrates, phosphates,
10 sulfates or any other post-translational modification.

The sample may be adsorbed on a probe under conditions which allow binding between the polypeptide and adsorbent material on the probe. The adsorbent material preferably comprises a metal chelating group complexed with a metal ion, and a
15 preferred metal is copper. Prior to detecting the polypeptide, unbound or weakly bound materials on the probe may be removed with a washing solution, thereby enriching the polypeptide in the sample. The sample is preferably adsorbed on a probe having an immobilised metal affinity capture (IMAC) surface capable of binding the polypeptide. The sample may be also adsorbed on a probe having
20 hydrophobic, strong anionic or weak cationic exchange surfaces under conditions which allow binding of the polypeptides. The probe may consist of a strip having several adsorbent wells, and be inserted into the spectrometer, then movable therein so that each well is in turn struck by the ionizing means (e.g. laser) to give a spectrometer reading. The polypeptide is preferably determined by surface-enhanced
25 laser desorption/ionisation (SELDI) and time of flight mass spectrometry (TOF-MS).

In principle, any body fluid can be used to provide a sample for diagnosis, but preferably the body fluid is cerebrospinal fluid (CSF); plasma, serum; blood; urine or
30 tears.

In one embodiment of the invention, the TSE is Creutzfeldt-Jakob disease (CJD). In this case, the polypeptide preferably has a molecular weight of about 4780, about 6700, about 8600 or about 13375, and the presence of one or more of such

polypeptides is indicative of CJD. Alternatively, one or more polypeptides having a respective molecular weight of about 3970, about 3990, about 4294, about 4478, about 10075, about 11730, about 14043 or about 17839 is determined, and the absence of one or more of such polypeptides is indicative of CJD. As a further
5 alternative, a polypeptide having a molecular weight of about 7770 is determined, and the presence of such polypeptide is indicative of CJD. According to one more example, CJD is indicated by a decrease in a peak at one or more of the following: about 3295, about 4315, about 4436, about 6200, about 8936, about 9107, about 9145, about 9185, about 9454 and about 13550 Da. According to yet a further example,
10 CJD is indicated by an increase in a peak at one or more of the following: about 7574, about 7930, about 7975 and about 8020. It will be appreciated that the invention embraces making a measurement at any one or more of the foregoing molecular weight values, in any combination thereof, for the purpose of making a diagnosis of CJD.

15

In another embodiment of the invention, the TSE is bovine spongiform encephalopathy (BSE). In this case, the polypeptide preferably has a molecular weight of about 10220, and the presence of the polypeptide is indicative of BSE.

20 In a further embodiment of the invention, the TSE is scrapie.

Measurement of the molecular weight of the polypeptide or polypeptides is effected in the mass spectrometer. All molecular weights herein are measured in Da. The molecular weights quoted above can be measured with an accuracy of better than 1%,
25 and preferably to within about 0.1%. The term "about" in connection with molecular weights in this specification therefore means within a variation of about 1%, preferably within about 0.1%, above or below the quoted value.

The invention also relates to the use of a polypeptide which is differentially contained
30 in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular weight in the range of from 3500 to 30000 and being determinable by mass spectrometry, for diagnostic, prognostic and therapeutic applications. This may involve the preparation and/or use of a material which recognizes, binds to or has

some affinity to the above-mentioned polypeptide. Examples of such materials are antibodies and antibody chips. The term "antibody" as used herein includes polyclonal antiserum, monoclonal antibodies, fragments of antibodies such as Fab, and genetically engineered antibodies. The antibodies may be chimeric or of a single species. The above reference to "prognostic" applications includes making a determination of the likely course of a TSE by, for example, measuring the amount of the above-mentioned polypeptide in a sample of body fluid. The above reference to "therapeutic" applications includes, for example, preparing materials which recognize, bind to or have affinity to the above-mentioned polypeptides, and using such materials in therapy. The materials may in this case be modified, for example by combining an antibody with a drug, thereby to target the drug to a specific region of the animal to be treated.

The methodology of this invention can be applied to the diagnosis of any TSE. Body fluid samples are prepared from infected and non-infected subjects. The samples are applied to a probe having a surface treated with a variety of adsorbent media, for differential retention of peptides in the sample, optionally using washing liquids to remove unbound or weakly bound materials. If appropriate, energy-absorbing material can also be applied. The probe is then inserted into a mass spectrometer, and readings are taken for the various sample/adsorbent combinations using a variety of spectrometer settings. Comparison of the infected and non-infected samples under a given set of conditions reveals one or more polypeptides which are differentially expressed in the infected and non-infected samples. The presence or absence of these polypeptides can then be used in the testing of a fluid sample from a subject under the same conditions (adsorbent, spectrometer settings etc.) to determine whether or not the subject is infected.

References herein to "presence or absence" of a polypeptide should be understood to mean simply that there is a significant difference in the amount of a polypeptide which is detected in the infected and non-infected sample. Thus, the "absence" of a polypeptide in a test sample may include the possibility that the polypeptide is actually present, but in a significantly lower amount than in a comparative test sample. According to the invention, a diagnosis can be made on the basis of the

presence or absence of a polypeptide, and this includes the presence of a polypeptide in a significantly lower or significantly higher amount with reference to a comparative test sample.

5 The following Examples illustrate the invention.

EXAMPLE 1

10 The objective of the present study was to detect specific polypeptides in body fluids (cerebrospinal fluid, plasma and others) of Creutzfeldt-Jacob affected patients. Samples were analysed by the Surface Enhanced Laser Desorption Ionization (SELDI) Mass Spectroscopy (MS) technology. This technology encompasses micro-scale affinity capture of proteins by using different types of retentate chromatography and then analysis by time of flight mass spectrometry. Different maps are thus
15 generated each corresponding to a typical protein profiling of given samples that were analysed with a Ciphergen Biosystem PBS II mass spectrometer (Freemont, CA, USA). Differential expressed peaks were identified when comparing spectra generated in a group of cerebrospinal fluid (CSF) samples from CJD-affected patients with a group of dementia-affected patients.

20 The SELDI analysis was performed using 2µl of crude human CSF samples in order to detect specific polypeptides with metal affinity. An immobilized copper affinity array (IMAC-Cu⁺⁺) was employed in this approach to capture proteins with affinity for copper to select for a specific subset of proteins from the samples. Captured
25 proteins were directly detected using the PBSII Protein Chip Array reader (Ciphergen Biosystems, Freemont, CA, USA).

The following protocol was used for the processing and analysis of ProteinChip arrays using Chromatographic TED-Cu(II) adsorbent array. TED is a
30 (tris(carboxymethyl)ethylencdiamine-Cu) adsorbent coated on a silicon oxide-coated stainless steel substrate.

- The surface was first loaded with 10 μ l of 100 mM copper sulfate to each spot and incubated for 15 minutes in a wet chamber.
- The chip was thereafter washed by two quick rinses with deionized water for about 10 seconds to remove the excess unbound copper.
- 5 ◦ Before loading the samples, the I-MAC 3 array was equilibrated once with 5 μ l of PBS NaCl 0.5 M for 5 minutes.
- After removing the equilibration buffer, 3 μ l of the same buffer were added before applying 2 μ l of CSF. The chip was incubated for 20 minutes in a wet chamber.
- 10 ◦ The samples were thereafter removed and the surface was washed three times with the equilibration buffer (5 minutes each).
- Two quick final rinses with water were performed.
- The surface was allowed to air dry, followed by the addition of 0.5 μ l of saturated sinapinic acid (SPA, Ciphergen Biosystem) prepared in 50%
15 acetonitrile, 0.5% trifluoroacetic acid.
- The chip was air dried again before analysis of the retained protein on each spot with laser desorption/ionization time-of-flight mass spectrometry.
- The protein chip array was inserted into the instrument and analysed once the appropriate detector sensitivity and laser energy have been established to
20 automate the data collection.
- The obtained spectra were analysed with the Biomark Wizard software (Ciphergen Biosystems, Freemont, CA, USA) running on a Dell Dimension 4100 PC. It generates consistent peak sets across multiple spectra.

- 25 Figures 1 to 4 shows the results of a comparative study which has been undertaken between CSF from CJD diagnosed patients and normal CSF, using the IMAC 3 protein chip array prepared as described above. In this study, we found that four peaks were significantly differentially increased in CSF from CJD affected patients. Their
molecular weights are respectively about 4780, 6700, 8600 and 13375 (mass accuracy
30 is around 0.1%). Figure 1 shows two spectral views, respectively of the normal and CJD sample, from 0 to 100,000 Da. Figure 2 shows the protein peak of 4780 Da, Figure 3 shows the protein peaks of 6700 and 8600 Da, and Figure 4 shows the

protein peak of 13375 Da. These data demonstrate that the peaks of about 4780, 6700, 8600 and 13375 Da can be used to diagnose CJD in CSF samples.

EXAMPLE 2

5

Example 1 was repeated using plasma samples from BSE-infected cattle (BSE +) and non-infected cattle (BSE -). The results are shown in Figures 5 and 6. Figure 5 shows a spectral view of each kind of sample from 0 to 50,000 Da. We observed that a protein around 10220 Da was significantly increased in BSE + plasma samples, as illustrated in Figure 6. This demonstrates that the peak of about 10220 Da can be used to diagnose BSE in plasma samples.

10

EXAMPLE 3

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Example 2 was repeated using plasma samples from CJD-infected patients (CJD +) and non-infected patients (CJD-, also referred to as CTS = Swiss Transfusion Centre). The results are shown in Figures 7 and 8. Figure 7 shows a spectral view of each kind of sample from 0 to 50,000 Da. We observed that polypeptides of about 3970, about 3990, about 4294, about 4478, about 10075, about 11730, about 14043 or about 17839 were significantly decreased in CJD + plasma samples, as illustrated in Figures 8A and B. We also observed that a peak of about 7770 Da was increased in CJD + plasma samples, as illustrated in Figure 8B. This demonstrates that the peak of about 3970, about 3990, about 4294, about 4478, about 10075, about 11730, about 14043, about 17839 or about 7770 Da can be used to diagnose CJD in plasma samples.

20

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EXAMPLE 4

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Example 3 was repeated, but using a more recent version of the software to analyse the data. The results are shown in Figures 9A to 9E, and indicate some new variations in protein levels, in addition to those identified in preceding Examples.

In Figure 9A, the arrow indicates a peak at about 3295 Da, which is decreased in the CJD samples.

In Figure 9B, the numbered arrows show the following:

- 1 - a peak at about 3976 Da, which is decreased in the CJD samples (corresponding to the 3970 Da peak in Example 3)
- 5 2 - a peak at about 3992 Da, which is decreased in the CJD samples (corresponding to the 3990 Da peak in Example 3)
- 3 - a peak at about 4300 Da, which is decreased in the CJD samples (corresponding to the 4294 Da peak in Example 3)
- 4 - a peak at about 4315 Da, which is decreased in the CJD samples
- 10 5 - a peak at about 4436 Da, which is decreased in the CJD samples
- 6 - a peak at about 4484 Da, which is decreased in the CJD samples (corresponding to the 4478 Da peak in Example 3)

In Figure 9C, the arrow indicates a peak at about 6200 Da, which is decreased in the CJD samples.

In Figure 9D, the numbered arrows show the following:

- 10 - a peak at about 7574 Da, which is increased in the CJD samples
- 11 - a peak at about 7773 Da, which is increased in the CJD samples (corresponding to the 7770 Da peak in Example 3)
- 20 12 - a peak at about 7930 Da, which is increased in the CJD samples
- 13 - a peak at about 7975 Da, which is increased in the CJD samples
- 14 - a peak at about 8020 Da, which is increased in the CJD samples
- 15 - a peak at about 8936 Da, which is decreased in the CJD samples
- 25 16 - a peak at about 9107 Da, which is decreased in the CJD samples
- 17 - a peak at about 9145 Da, which is decreased in the CJD samples
- 18 - a peak at about 9185 Da, which is decreased in the CJD samples
- 19 - a peak at about 9454 Da, which is decreased in the CJD samples

In Figure 9E, the numbered arrows show the following:

- 20 - a peak at about 10068 Da, which is decreased in the CJD samples (corresponding to the 10075 Da peak in Example 3)
- 21 - a peak at about 13550 Da, which is decreased in the CJD samples

22 - a peak at about 17809 Da, which is decreased in the CJD samples (corresponding to the 17839 Da peak in Example 3)

5 This Example demonstrates that any one of the above peaks, or more than one of them in any combination, can be used to diagnose CJD.

* * * * *

10 Each of the above cited publications is herein incorporated by reference to the extent to which it is relied on herein.

CLAIMS

1. A method of diagnosis of a transmissible spongiform encephalopathy (TSE) or
5 the possibility thereof in a subject suspected of suffering from the TSE, which
comprises subjecting a sample of body fluid taken from the subject to mass
spectrometry, thereby to determine a test amount of a polypeptide in the sample,
wherein the polypeptide is differentially contained in the body fluid of TSE-infected
10 subjects and non-TSE-infected subjects, and has a molecular weight in the range of
from 3500 to 30000; and determining whether the test amount is consistent with a
diagnosis of TSE.
2. A method according to Claim 1, in which the polypeptide is present in the
body fluid of TSE-infected subjects and not present in the body fluid of non-TSE-
15 infected subjects, whereby the presence of the polypeptide in a body fluid sample is
indicative of TSE.
3. A method according to Claim 1, in which the polypeptide is not present in the
body fluid of TSE-infected subjects and present in the body fluid of non-TSE-infected
20 subjects, whereby the non-presence of the polypeptide in a body fluid sample is
indicative of TSE.
4. A method according to any of Claims 1 to 3, in which the mass spectrometry
is laser desorption/ionization mass spectrometry.
25
5. A method according to any of Claims 1 to 4, in which the sample is adsorbed
on a probe having an immobilised metal affinity capture (IMAC), hydrophobic, strong
anionic or weak cationic exchange surface capable of binding the polypeptide.
- 30 6. A method according to any of Claims 1 to 5, in which the polypeptide is
determined by surface-enhanced laser desorption/ionisation (SELDI) and time of
flight mass spectrometry (TOF-MS).

7. A method according to any of Claims 1 to 6, in which the body fluid is cerebrospinal fluid, plasma, serum, blood or tears.

8. A method according to any of Claims 1 to 7, in which a plurality of peptides is
5 determined in the sample.

9. A method according to any of Claims 1 to 8, in which the TSE is Creutzfeldt-Jakob disease (CJD).

10. A method according to Claim 9, in which one or more polypeptides having a
10 respective molecular weight of about 4780, about 6700, about 8600 or about 13375 is determined, and the presence of one or more of such polypeptides is indicative of CJD.

11. A method according to Claim 9 or 10, in which one or more polypeptides
15 having a respective molecular weight of about 3970, about 3990, about 4294, about 4478, about 10075, about 11730, about 14043 or about 17839 is determined, and the absence of one or more of such polypeptides is indicative of CJD.

12. A method according to any of Claims 9 to 11, in which a polypeptide having a
20 molecular weight of about 7770 is determined, and the presence of such polypeptide is indicative of CJD.

13. A method according to any of Claims 9 to 12, in which a polypeptide having a
25 molecular weight of about 3295, about 4315, about 4436, about 6200, about 8936, about 9107, about 9145, about 9185, about 9454 or about 13550 Da is determined, and the absence or decreased amount of one or more of such polypeptides is indicative of CJD.

14. A method according to any of Claims 9 to 13, in which a polypeptide having a
30 molecular weight of about 7574, about 7930, about 7975 or about 8020 Da is determined, and the presence or increased amount of one or more of such polypeptides is indicative of CJD.

15. A method according to any of Claims 1 to 8, in which the TSE is bovine spongiform encephalopathy (BSE).
- 5 16. A method according to Claim 15, in which the polypeptide has a molecular weight of about 10220, and the presence of the polypeptide is indicative of BSE.
17. A method according to any of Claims 1 to 8, in which the TSE is scrapie.
- 10 18. Use of a polypeptide which is differentially contained in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular weight in the range of from 3500 to 30000 and being determinable by mass spectrometry, for diagnostic, prognostic and therapeutic applications.
- 15 19. Use for diagnostic, prognostic and therapeutic applications of a material which recognizes, binds to or has affinity for a polypeptide which is differentially contained in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular weight in the range of from 3500 to 30000 and being determinable by mass spectrometry.
- 20 20. Use according to Claim 19, in which the material is an antibody or antibody chip.
21. A kit for use in diagnosis of TSE, comprising a probe for receiving a sample of
25 body fluid, and for placement in a mass spectrometer, thereby to determine a test amount of a polypeptide in the sample, wherein the polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects, and has a molecular weight in the range of from 3500 to 30000.
- 30 22. A kit according to Claim 21, in which the probe contains an adsorbent for adsorption of the polypeptide.

23. A kit according to Claim 22, further comprising a washing solution for removal of unbound or weakly bound materials from the probe.

ABSTRACT

DIAGNOSTIC METHOD FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

5

Transmissible spongiform encephalopathy (TSE) is diagnosed in a subject by using mass spectrometry to observe a polypeptide in a sample of body fluid taken from the subject. The polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-infected subjects, and has a molecular weight in the range of from

10

3500 to 30000.

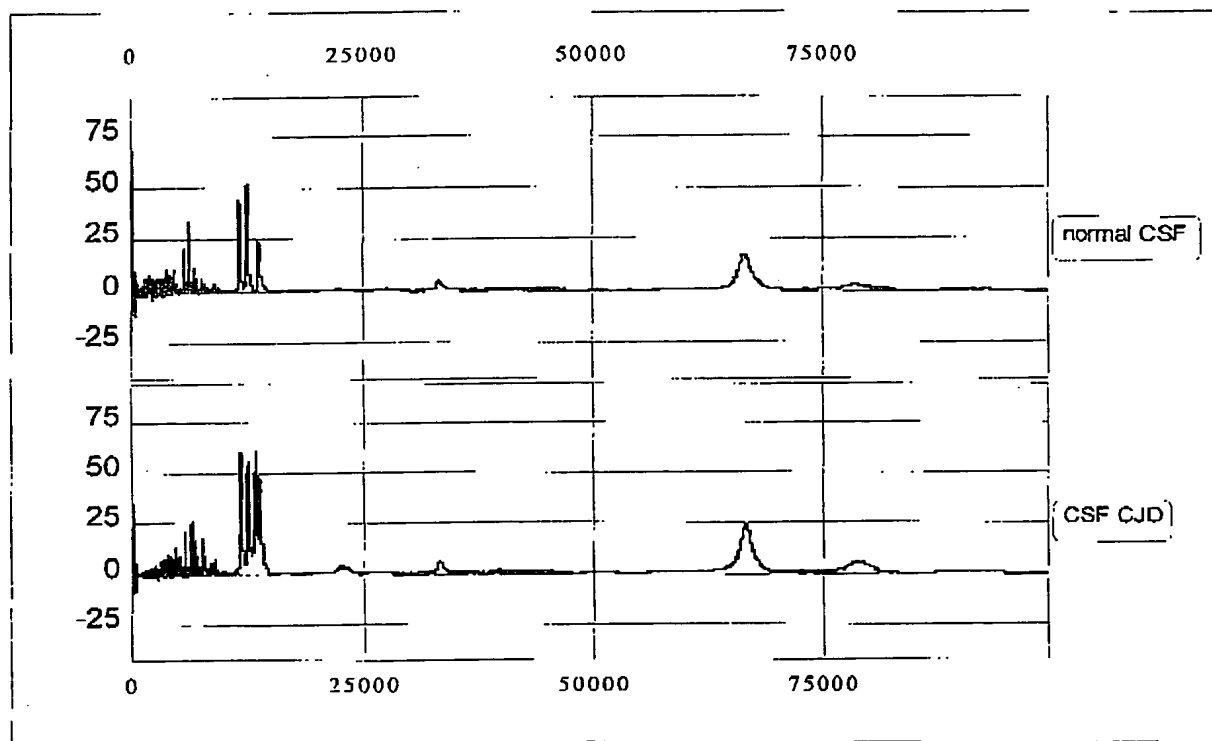


Figure 1

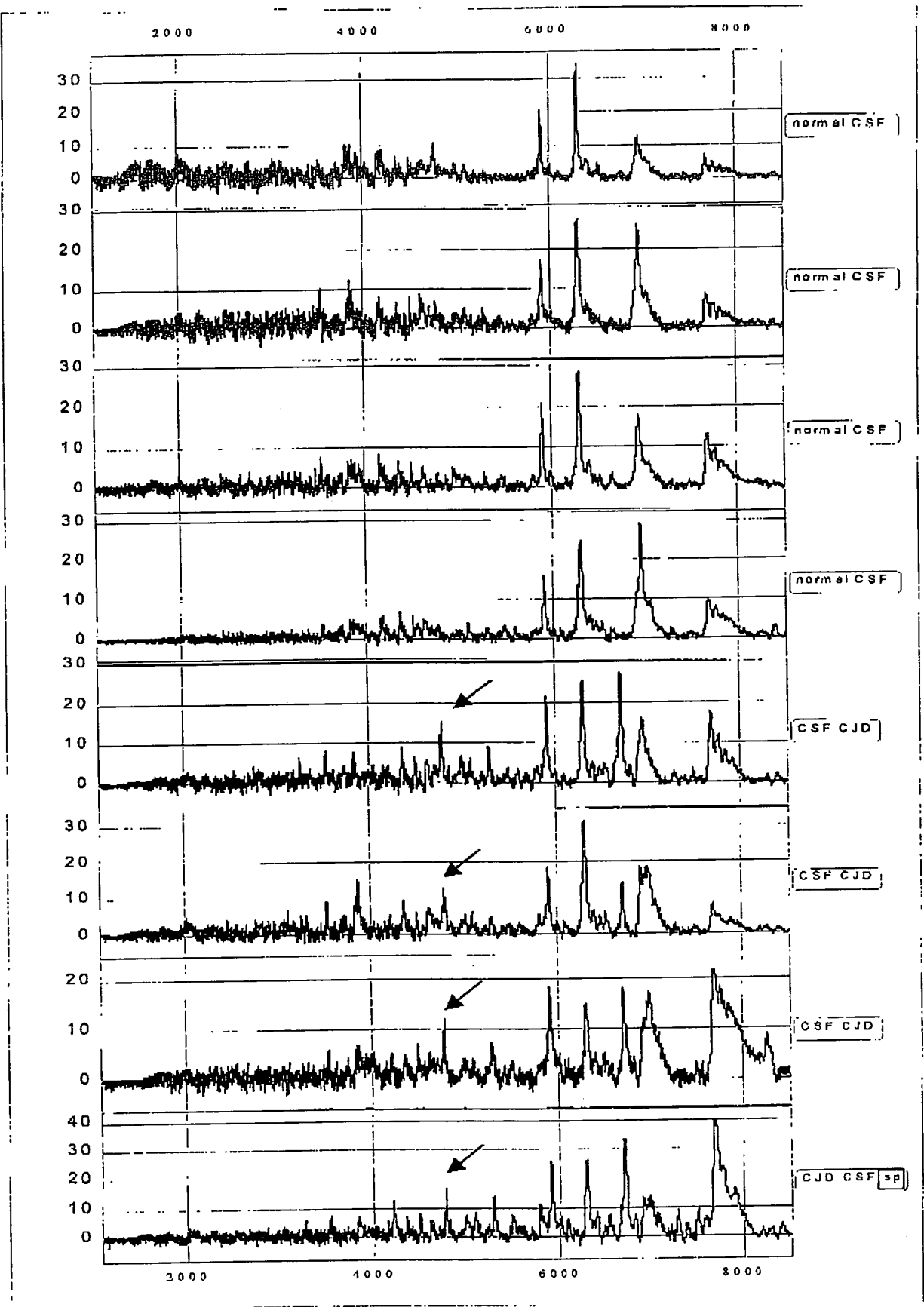


Figure 2

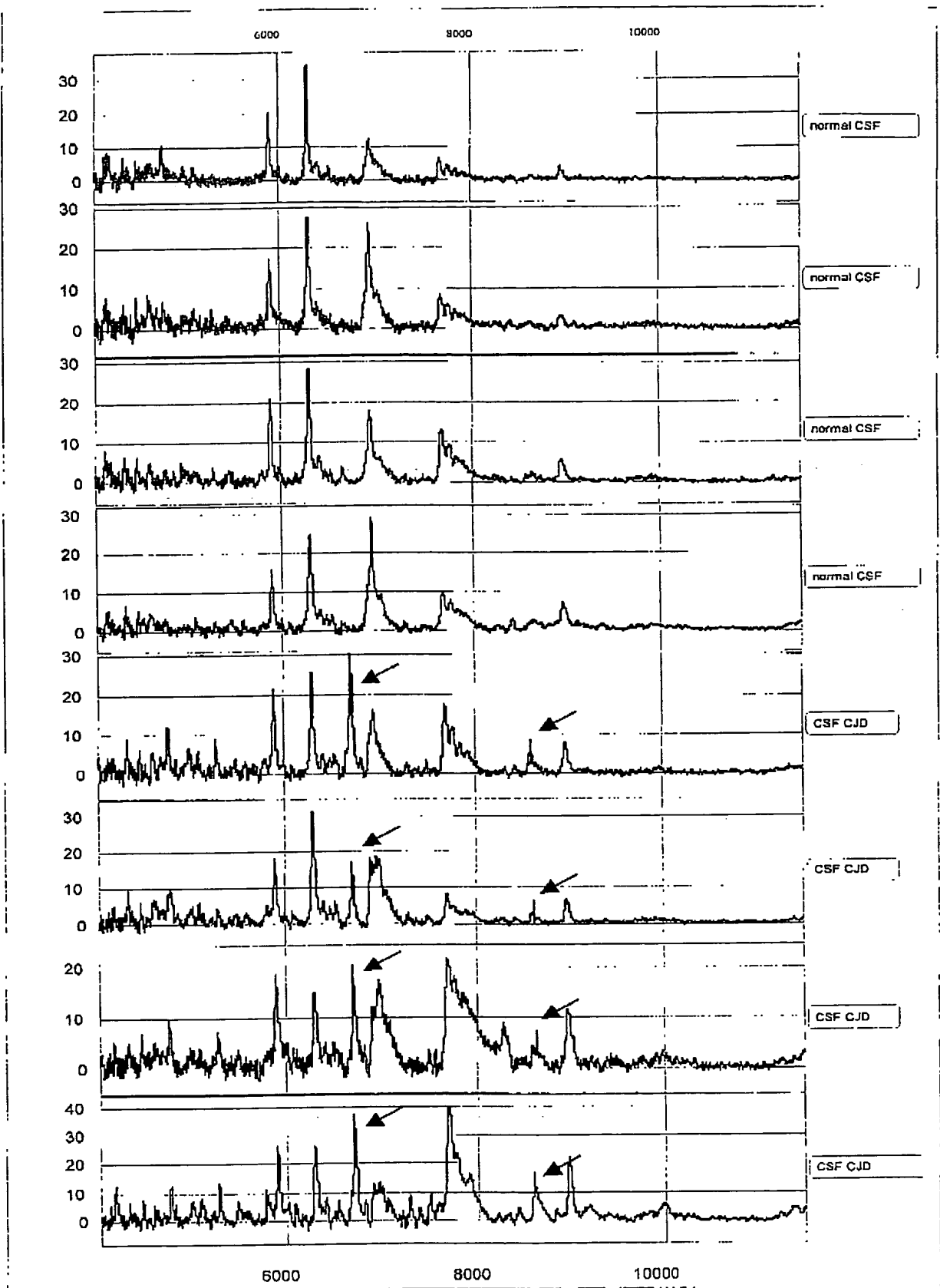


Figure 3

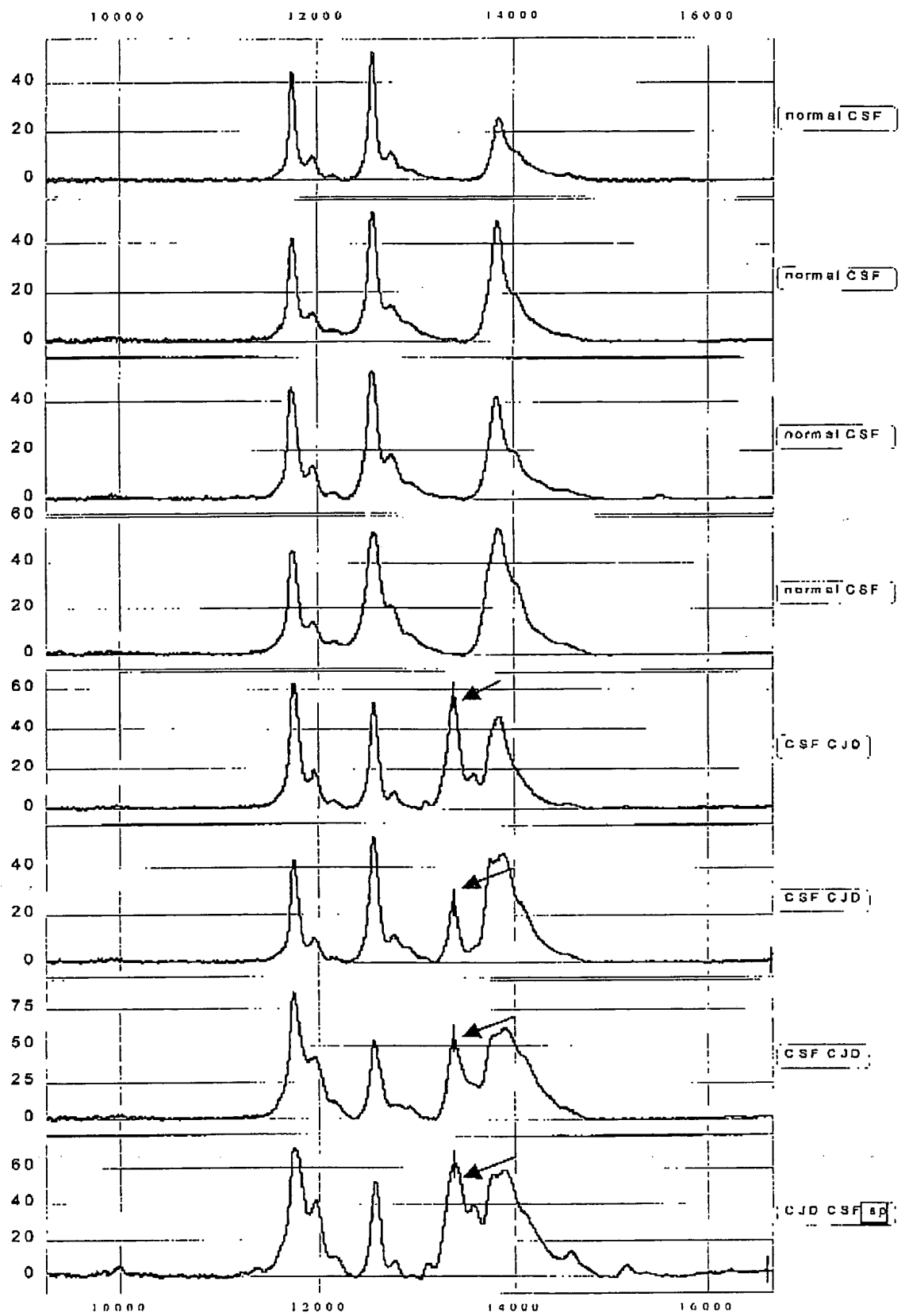


Figure 4

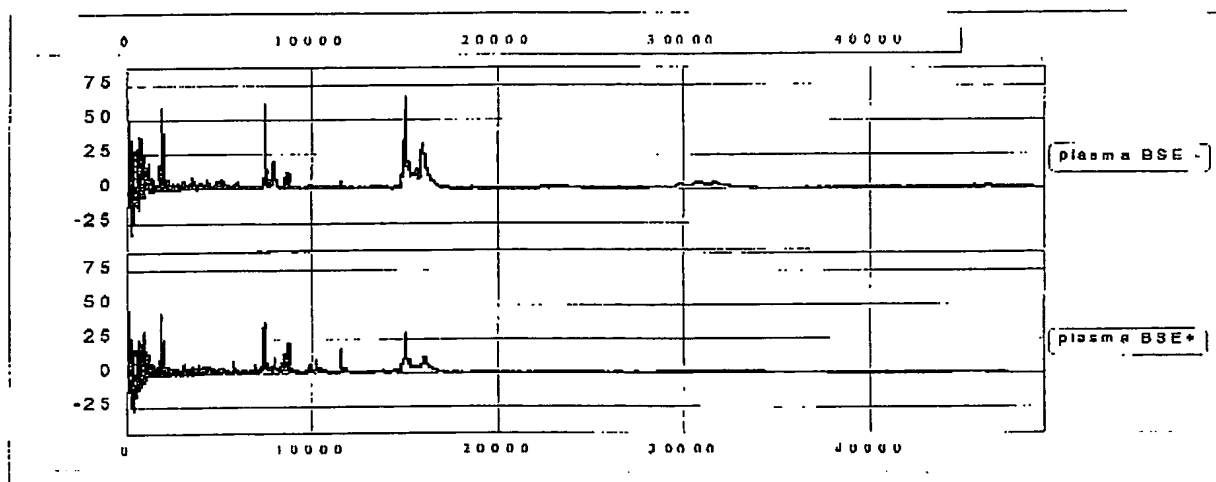


Figure 5

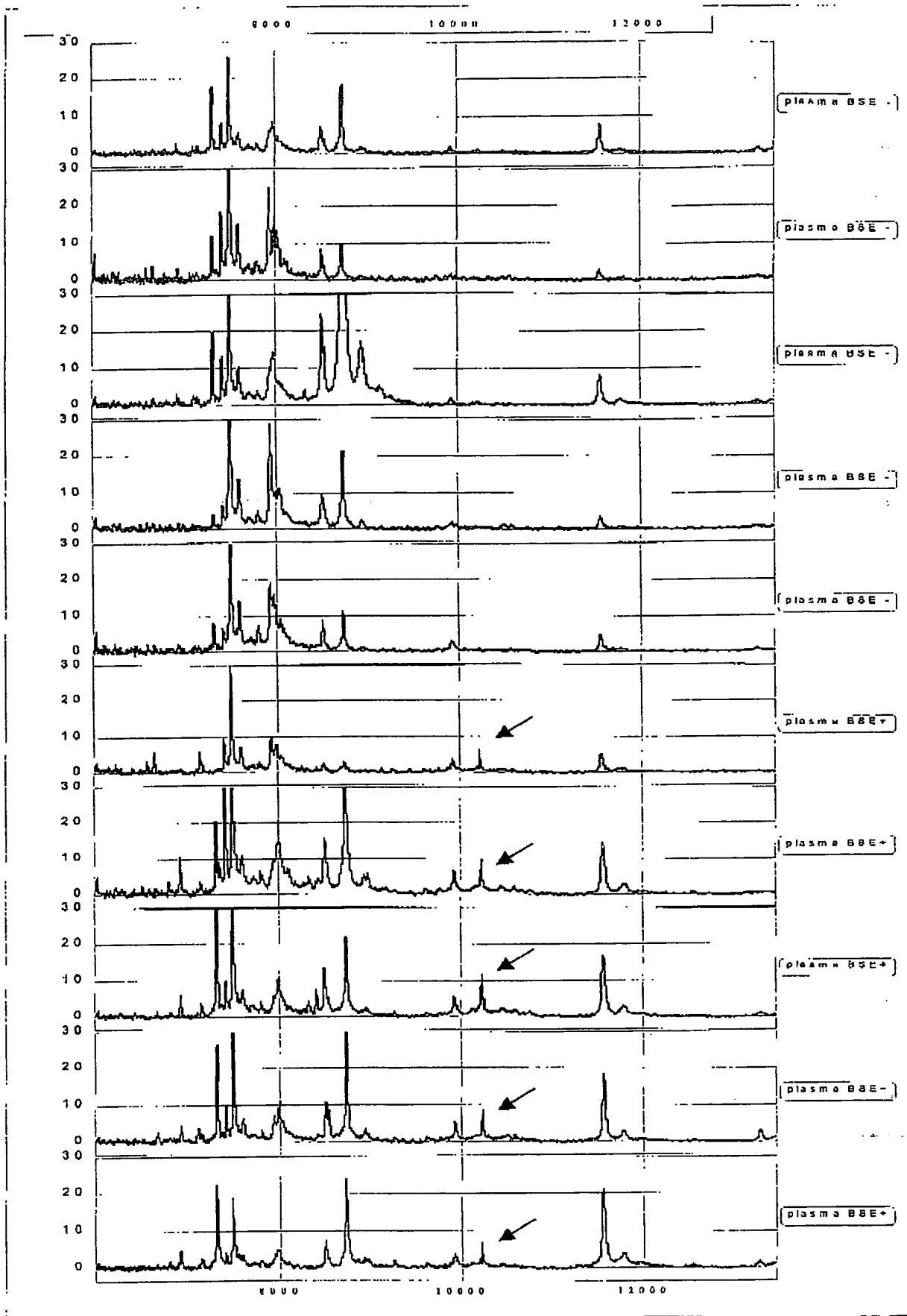


Figure 6

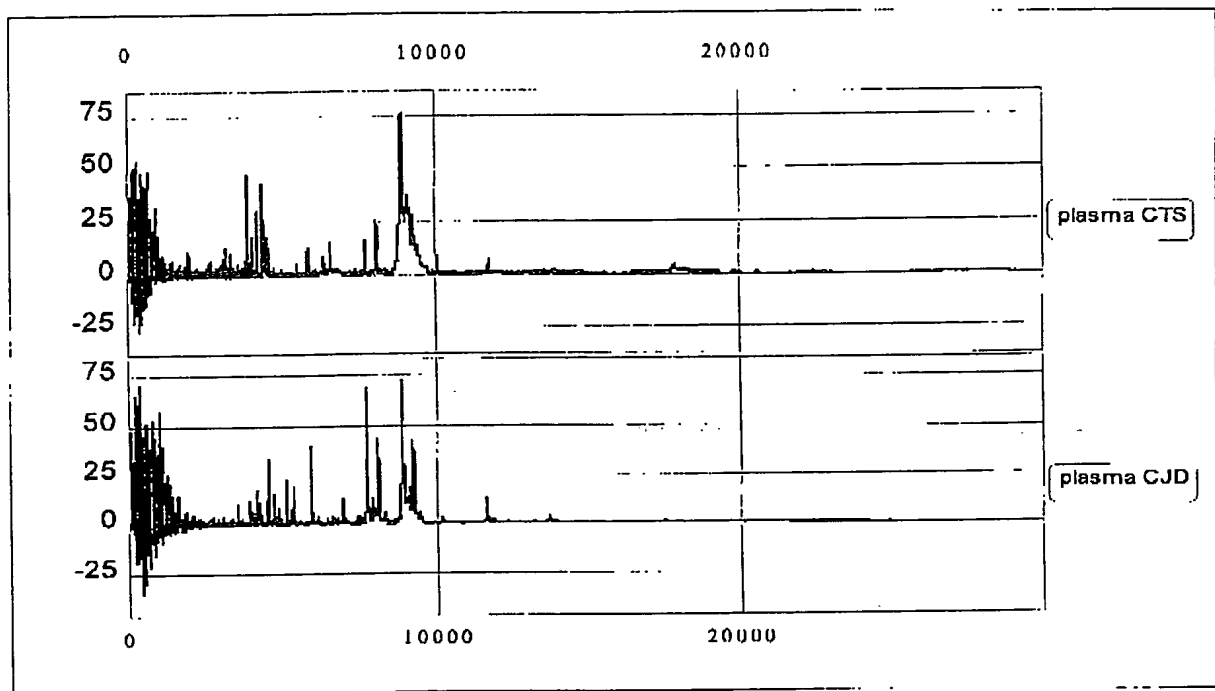


Figure 7

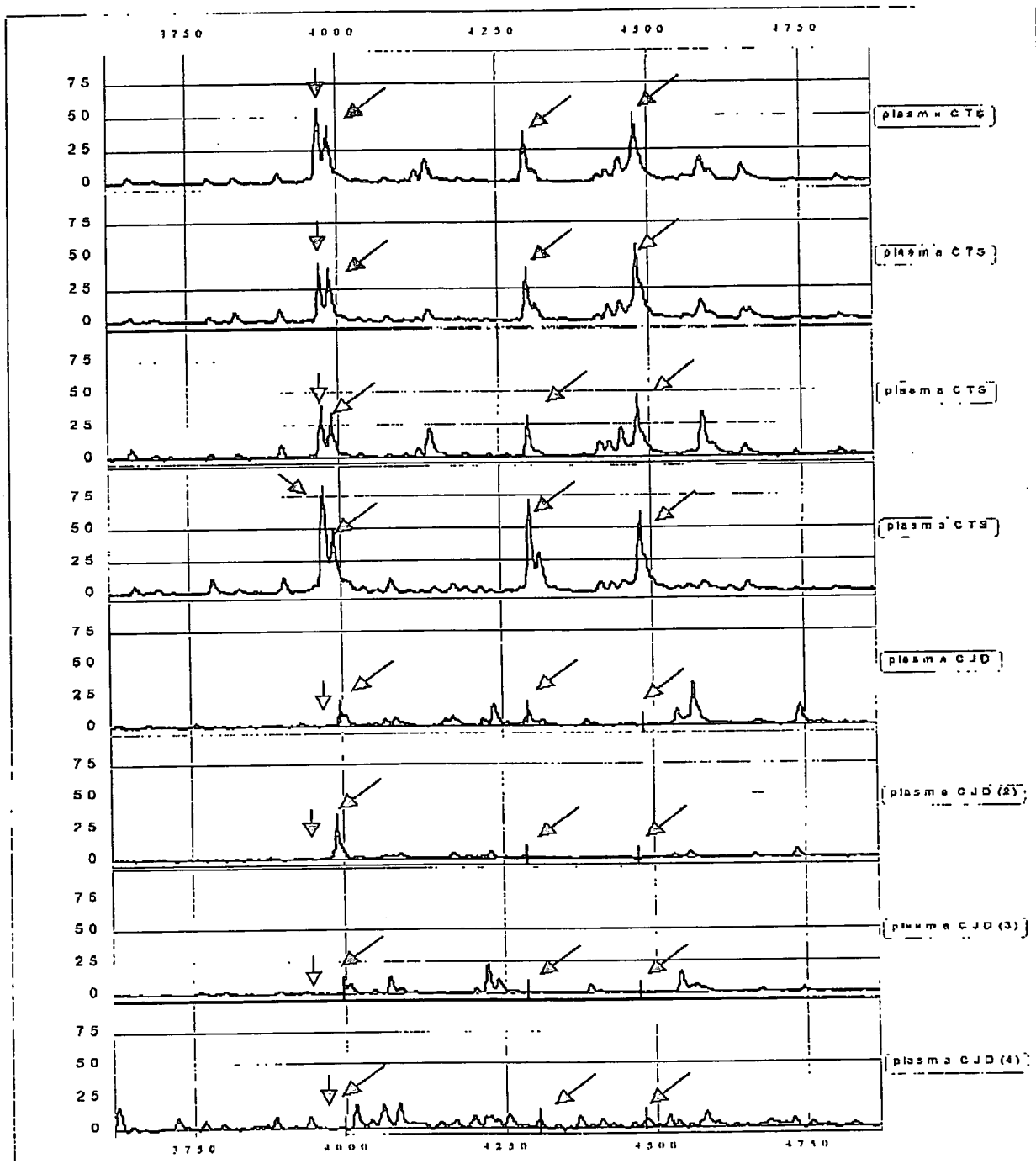


Figure 8A : 3970, 3990, 4294, 4478

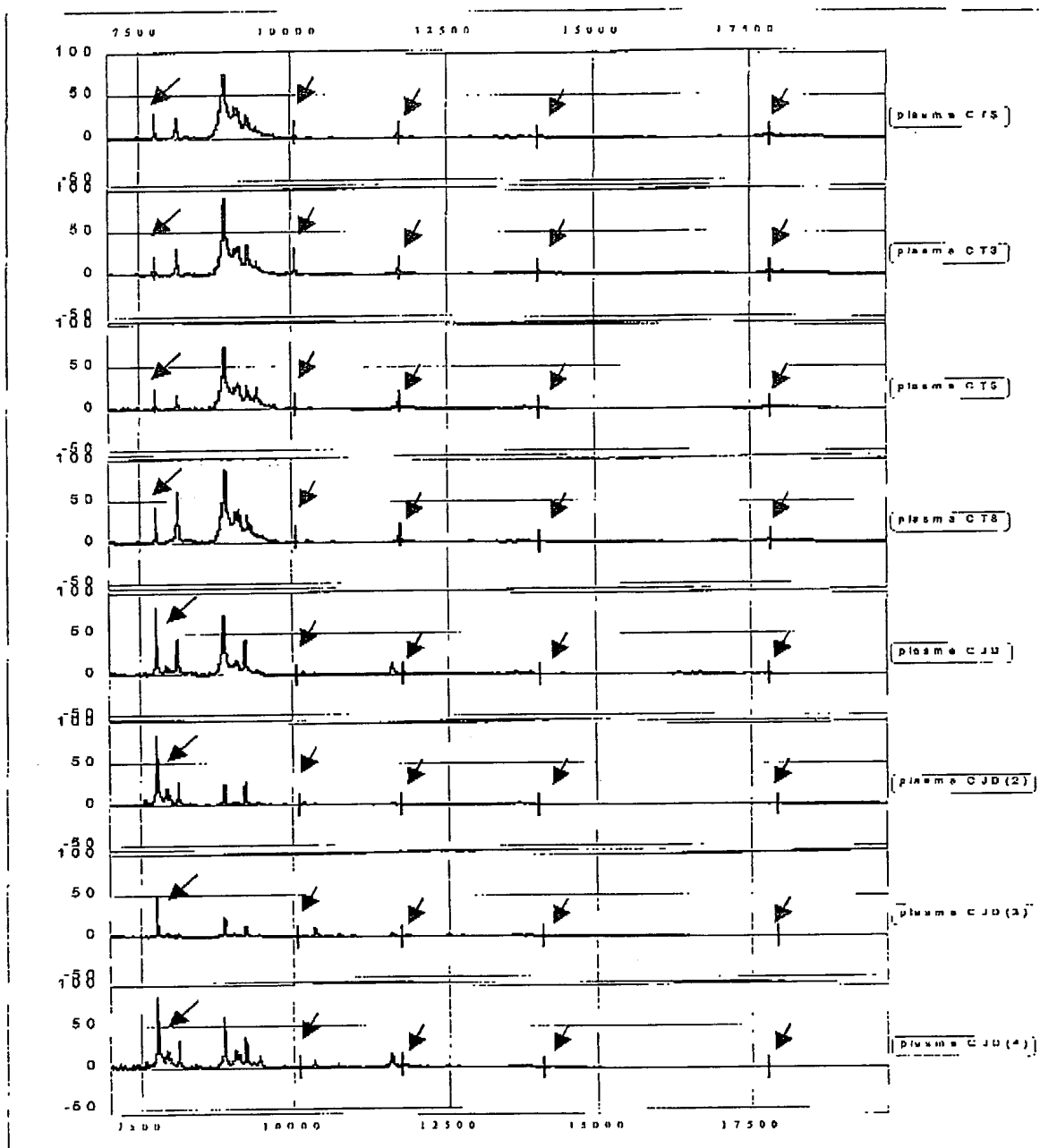


Figure 8B : 7770, 10075, 11730, 14043, 17839

Figure 9A

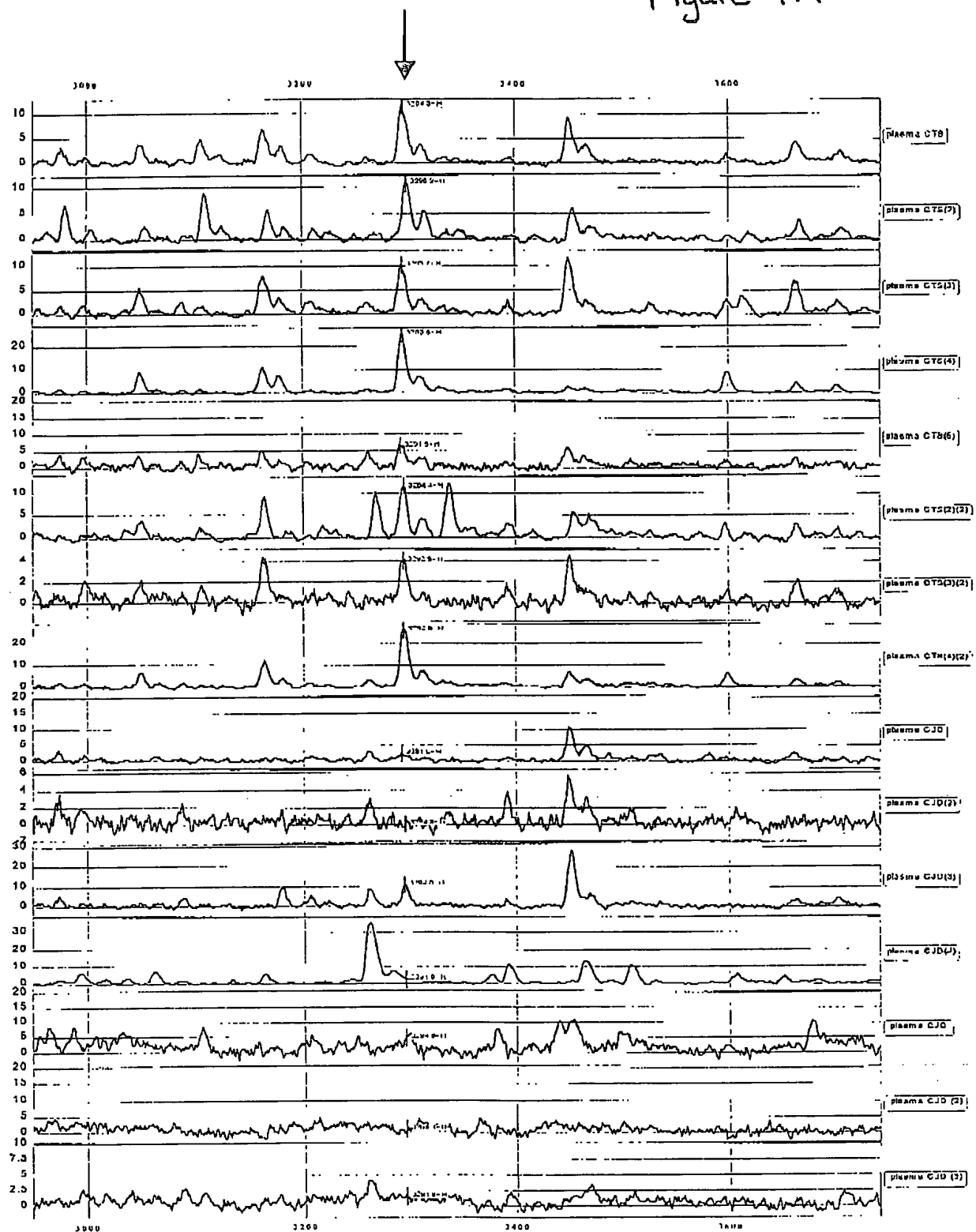


Figure 9B

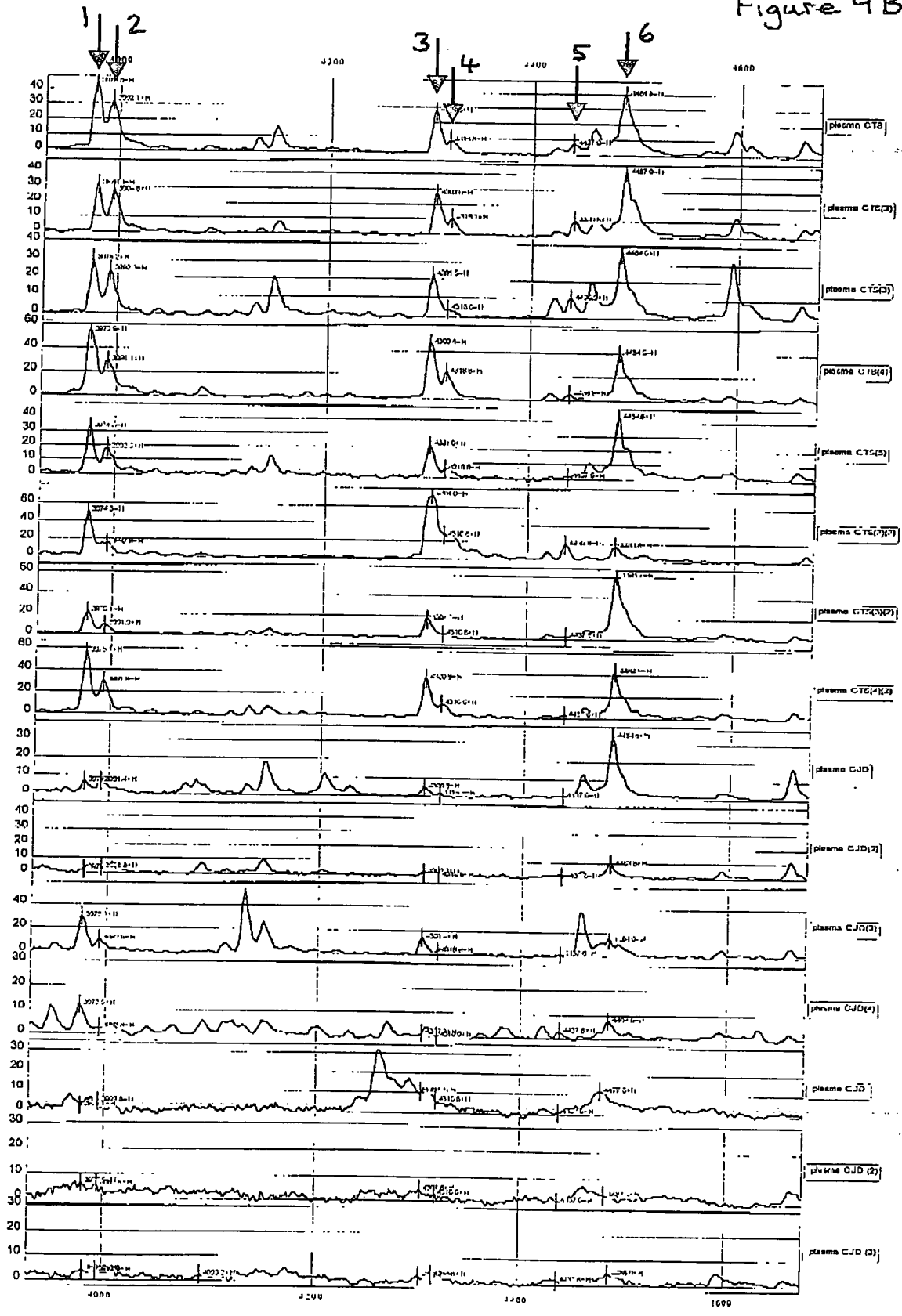


Figure 9C

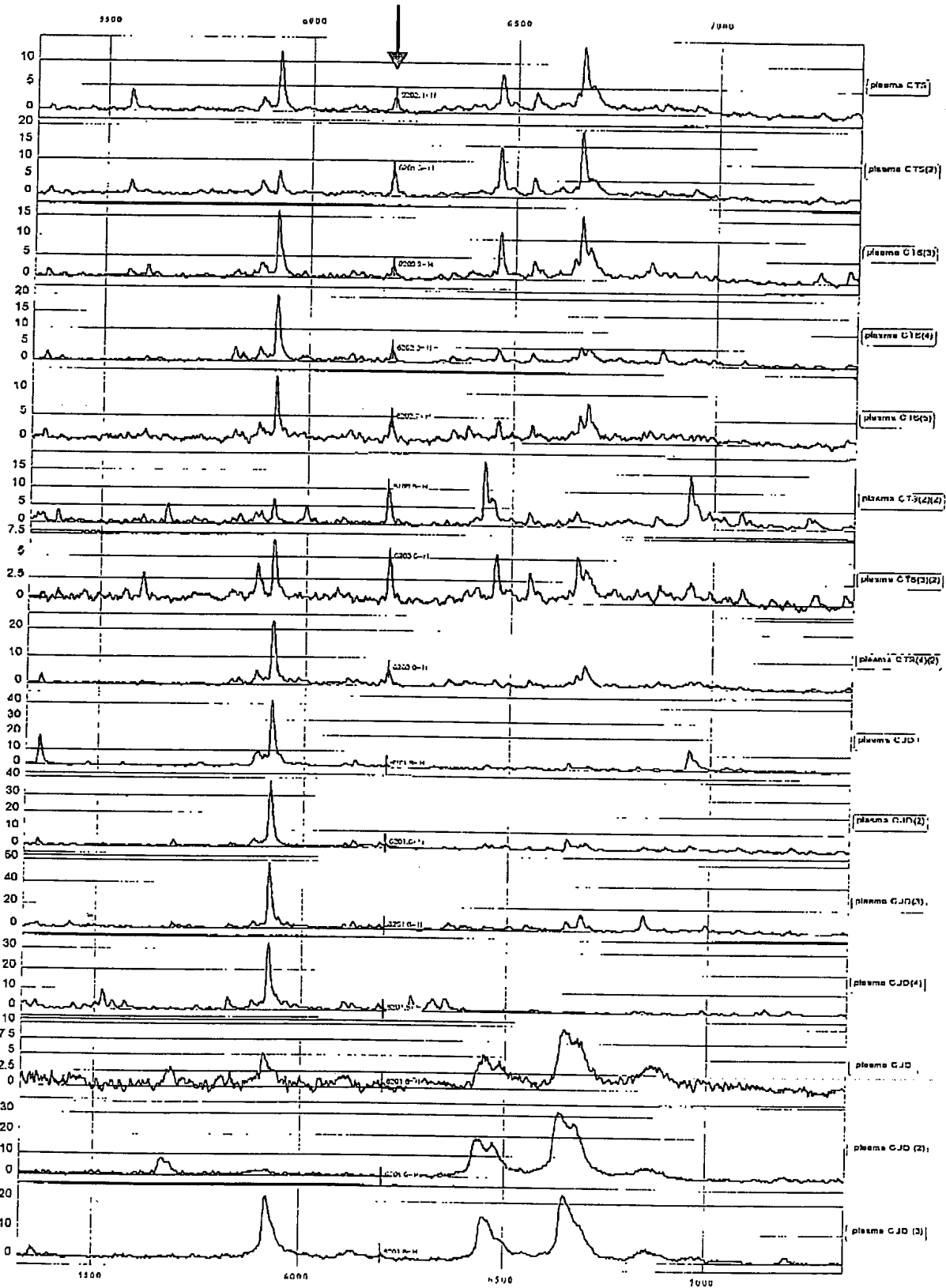


Figure 9D

